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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/506,958	05/02/2005	Helen Braven	ATLAS 8095 US	8800
39843	7590	05/21/2009		
BELL & ASSOCIATES 58 West Portal Avenue No. 121 SAN FRANCISCO, CA 94127			EXAMINER POHNERT, STEVEN C	
			ART UNIT 1634	PAPER NUMBER
			MAIL DATE 05/21/2009	DELIVERY MODE PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 10/506,958	Applicant(s) BRAVEN ET AL.	
	Examiner STEVEN C. POHNERT	Art Unit 1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 27 February 2009.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-9, 11-25, 43-45, 91-105 and 109-133 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1-9, 11-25, 43-45, 91-105 and 109-133 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☒ The drawing(s) filed on 07 September 2004 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☒ All b) ☐ Some * c) ☐ None of:
1. ☒ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
- * See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413) |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date. _____ |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

This action is in response to amendment of 2/27/2009.

Claims 26-42, 46-90, 106-108 are canceled.

Claims 1-9, 11-25, 43-45, 91-105, 109-133 are being examined.

This action is FINAL.

Claim Rejections - 35 USC § 103

1. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

2. Claims 1-9, 11-18, 20-25, 43-45, 91-101, 109-116, 119-129 are rejected under 35 U.S.C. 103(a) as being unpatentable over Calzone et al (Methods in Enzymology (1987) volume 152, pages 611-632) in view of Clinical Micro Sensors, Inc (WO01/06016, Published January 25, 2001).

Clinical Micro Sensors is here after referred to as CMS.

With regards to claim 1 and 109, Calzone teaches a method of mapping gene transcripts by nuclease protection assay to determine the size and length of the nucleic acid (see title, figure 1). Figure 1 teaches the labeled probes are hybridized and then digested by S1 nuclease or Exonuclease. Calzone teaches the digest complex is run on a gel allowing for determination of size and characteristics of the probe and thus the nucleic acid.

With regards to claim 2 and 112, the presence of the non-degraded probe of Calzone teaches the presence of the nucleic acid.

With regards to claim 3, 94 and 113, Calzone teaches in figure 2 the relative portions of degraded and non-degraded probes as the 520 base primary transcript and the 75 base mature mRNA.

With regards to claim 4, Calzone teaches the use of nucleases that selectively digest single strand nucleic acid (see figure 1).

With regards to claim 5, 97 Calzone teaches the use of mung bean endonuclease (see page 612, 1st paragraph).

With regards to claim 6, 96 Calzone teaches the use of RNase A to digest excess RNA (page 620, 1st paragraph).

With regards to claims 7, 8, 98 Calzone teaches the use of S1, which is a DNase (see figure 1).

With regards to claim 9, 95 Calzone teaches the use of exonuclease VII (see figure 1).

With regards to claim 11, Calzone teaches the use of nucleases that selectively digest free strands of double stranded hybridized duplexes. Thus the selective degradation of non-hybridized segments of the hybridized nucleic acid is selective digesting at least one strand.

With regards to claim 12 and 110-111, Calzone teaches that exonuclease VII hydrolyzes single stranded DNA in the 5' direction (page 612, 1st paragraph). Calzone thus teaches an enzyme that is a 5' nuclease.

Calzone does not teach an electrochemically active marker. Calzone does not teach a 5' nuclease that is a polymerase (claim 13). Calzone does not teach a thermostable 5' nuclease/DNA polymerase (claims 14-15). Calzone does not teach detection of mutations, polymorphisms, or quantitation of nucleic acids (claims 20-24, 43, 119-123). Calzone does not teach software for use in the method of detecting of pathogens (claims 25 and 44-45). Calzone does not teach the use of 2 probes with different electrochemical labels, or detection by voltametry, amperometry, or differential pulse voltametry (claims 91, 92, 99-101, 125-127).

However, Clinical Micro Sensors (CMS) teaches the use of electrochemical detection moieties (CMS calls ETMs) to as labels to detect nucleic acid sequences (see abstract). CMS further that ETMs allows amplification of signal resulting in sensitive assays (see page 54, lines 7-50). CMS teaches, "Thus, the present invention provides for extremely specific and sensitive probes, which may, in some embodiments, detect target sequences without removal of unhybridized probe. This will be useful in the generation of automated gene probe assays" (page 114). CMS teaches these labels and probes allow for automation (page 114).

With regards to claims 11 and 12, CMS teaches the use of Invadertm technology as a preferred embodiment. CMS teaches the use of an "invader primer and a signaling primer that has an overlapping sequence(see page 42, lines 8-11). CMS further teaches that invader technology is based on structure specific polymerases that cleave nucleic acids in a site-specific manner (see page 42, lines 1-3). With regards to claim 12, CMS teaches the use of 5' thermostable nucleases (see page 42, line 16).

With regard to claim 13, CMS further teaches that invader technology is based on structure specific polymerases that cleave nucleic acids in a site-specific manner (see page 42, lines 1-3).

With regards to claims 14, 15, and 114-115, CMS further teaches this polymerase/nuclease can be from Taq (see page 42, line 16).

With regards to claims 16, and 124, CMS teaches a new primer binds after cleavage (see page 42, line 19). CMS thus teaches a solution comprises a primer pair suitable for extension.

With regards to claim 17, 115, 116 CMS teaches PCR amplification using Taq polymerase by cycling in the preferred embodiment (see page 21, lines 16-20).

With regards to claim 18, CMS teaches the use of invader technology using two probes. Invader technology is based on hybridization of first oligonucleotide and a second oligonucleotide to a target sequence, with a non-complementary overlap that is cleaved by a nuclease (see page 42, lines 1-25). CMS further the ETM tagged tail is released in the cleavage by a nuclease that specifically recognizes the structure of the 2 probe target complex, the cleavage releasing the tail with an ETM tag (see page 42, lines 1-5 and lines 21-25). This cleavage shortens the oligonucleotide to which the ETM is attached.

With regards to claims 20, 119, and 121 CMS teaches the detection of mutations, which are nucleic acid polymorphisms (see page 113, line 12).

With regards to claims 21, 43, and 120, CMS teaches the detection of BRCA1, P53, APOE4 for the presymptomatic screening of patients. CMS thus teaches detection of allelic polymorphisms (see page 113, line 12).

With regards to claim 22, CMS teaches the probe array for use in sequencing by hybridization which would determine single nucleotide polymorphisms (see page 113, line 33).

With regards to claim 23, 122, CMS teaches its method allows detection of 10^6 molecules (see page 114, line 35). CMS thus teaches the quantifiable detection of nucleic acid species.

With regards to claim 24, 123, CMS teaches its method can be used for the detection of mRNA (see page 114, lines 18).

With regards to claim 25, CMS teaches the use of software directed microprocessor for the detection of electrochemical active species (figure 20A).

With regards to claims 44 and 45, CMS teaches the detection of pathogens in a sample. CMS teaches, "The detection of specific nucleic acids is an important tool for diagnostic medicine and molecular biology research. Gene probe assays currently play roles in identifying infectious organisms such as bacteria and viruses, in probing the expression of normal genes and identifying mutant genes such as oncogenes, in typing tissue for compatibility preceding tissue transplantation, in matching tissue or blood samples for forensic medicine, and for exploring homology among genes from different species" (page 2, page 113). CMS thus teaches methods of detecting pathogens and therapy response (compatibility testing prior to transplantation).

With regards to claims 91 and 125 CMS teaches in figure 32 the use of two oligonucleotide probes 10 and 12 with two different EMT probes 135 and 13.

With regards to claims 92, 126, and 127, CMS teaches in figure 20 a-o, that two or labels can be distinguished by peaks on their voltametric traces.

With regards to claim 99, CMS teaches the use of voltammetry methods for detection (see page 105, line 10).

With regards to claims 100 and 128 CMS teaches the use of amperometry for detection (see page 105, line 10).

With regards to claims 101 and 129 CMS teaches the use of differential pulse voltametry (see page 106, lines 25-26).

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve the method of Calzone by use of the ETM labels and nucleases and methods of detecting ETM labels taught by CMS. The artisan would be motivated to substitute one known method of labeling (radioactive) for another method of labeling nucleotides (ETM). The artisan would be motivated to substitute the ETM label of CMS for the radioactive labels of Calzone, because the ETM labels eliminate the use of radioactivity and the hazards associated with the risk and because CMS teaches the ETM allows amplification of the signal and sensitive detection of target sequences. The artisan would be motivated to substitute the nucleases of CMS for those of Calzone as the nucleases of Calzone are substitutes and CMS teaches they will work. The artisan would have a reasonable expectation of

success by the combination of Calzone and CMS as they are both methods of detecting nucleic acids using nucleases and have been demonstrated to work.

Response to Arguments

The response traverses the instant rejection asserting that neither Calzone nor CMS teach a method comprising determining information relating to the electrochemically active marker which correlates with the size and characteristics of the degraded or non-degraded oligonucleotide probes. These arguments have been thoroughly reviewed but are not considered persuasive as neither the specification nor claims set forth a limiting definition of "size and characteristics of degraded or non-degraded probes". Thus the teachings of CMS that the probes were detected by hybridization teaches the probes were present and of a size sufficient to allow hybridization and are characterized as not-degraded or alternatively characterized by the sequence required allowing hybridization.

The response asserts that one of skill in the art would have not combined the teachings of Calzone with the teachings with teachings of CMS to come up with the electrochemical detection of molecules claimed. This argument has been thoroughly reviewed but is not considered persuasive as the artisan would merely be substituting the ETM labeling and detection method of CMS in the nuclease assay of Calzone. The teachings of Calzone and CMS are both drawn to detection of nucleic acids by structure specific nucleic acids. Further these appear to be arguments of counsel that have not been supported by evidence.

The response directs the examiners attention to the teachings of page 5, lines 11-15, ""(t)he present invention is based upon the observation that an electrochemically active marker such as metallocene exhibits different electrochemical characteristics depending upon whether or not it is attached to a nucleotide, whether or not that nucleotide is incorporated into an oligonucleotide or not, and the length of any such oligonucleotide". Applicants submit that this property would not have been predicted by one of skill in the art and was unexpected". These arguments have been thoroughly reviewed but are not considered persuasive as first they are arguments of counsel that has not been supported by evidence. In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., the present invention is based upon the observation that an electrochemically active marker such as metallocene exhibits different electrochemical characteristics depending upon whether or not it is attached to a nucleotide, whether or not that nucleotide is incorporated into an oligonucleotide or not, and the length of any such oligonucleotide) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). The examiner notes that amendment of the claims to incorporate the limitations suggested by the cited portion of page 5, may cause the instant rejection to be withdrawn, but would require further search and consideration.

The response further asserts that Calzone does not teach the use of oligonucleotide probes (bottom page 12-13). These arguments have been thoroughly

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reviewed but are not considered persuasive as the specification does not set forth a limiting definition of oligonucleotide. Thus oligonucleotide is being the broadest reasonable interpretation of an RNA or DNA sequence of two or more covalently linked nucleotides. The probes of Calzone are 2 or more covalently linked oligonucleotides, which are defined by CMS to be oligonucleotides (page 18, last paragraph).

The response (page 13) then presents the teachings of Calzone to the length of the probe-target hybrid possible contaminants and degradation of the probes over time and then asserts that Calzone teaches away from using oligonucleotide probes. These arguments have been thoroughly reviewed but are not considered persuasive as Calzone teachings give guidance and direction to the artisan on these issues, but do not discourage the use of these probes. Calzone teaches the use of the probes and thus contrary to the assertion does not teach away. Calzone demonstrates that one of ordinary skill in the art at the time the invention was made could predictably use these probes.

It appears to the response is attempting to distinguish the probes used by Calzone from the "oligonucleotide probes" recited in the claimed invention, but that there is no structural limitation in the claims that makes this distinction. As noted by applicant, the "probes" used by Calzone fall within the broadest reasonable interpretation of the claims.

The response asserts that CMS does not teach a method comprising determining information relating to the electrochemically active marker which correlates with the size and characteristics of the degraded or non-degraded oligonucleotide probes. These

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arguments have been thoroughly reviewed but are not considered persuasive as neither the specification nor claims set forth a limiting definition of "size and characteristics of degraded or non-degraded probes". Thus the teachings of CMS that the probes were detected by hybridization teaches the probes were present and of a size sufficient to allow hybridization and are characterized as not-degraded or alternatively characterized by the sequence required allowing hybridization. Thus the electrochemical hybridization of CMS correlates to size and characteristics of the (degraded or non-degraded) probe.

The response continues by asserts that CMS teaches that uncleaved probes are removed and therefore the non-degraded probes of CMS cannot contribute to the detection of the probe sequence. These arguments have been thoroughly reviewed but are not considered persuasive as the claims are drawn to the electrochemical marker correlating to the size and characteristics of the degraded or non-degraded probes, but do not require both, contrary to the assertion of the response.

The response continues by asserting that there is no evidence that one of skill in the art would be motivated to consider the modification of Calzone by the ETM labels of CMS. KSR forecloses the argument that a specific teaching, suggestion or motivation is required to support obviousness. Further the rejection stated, "The artisan would be motivated to substitute one known method of labeling (radioactive) for another method of labeling nucleotides (ETM). The artisan would be motivated to substitute the ETM label of CMS for the radioactive labels of Calzone, because the ETM labels eliminate the use of radioactivity and the hazards associated with the risk and because CMS

teaches the ETM allows amplification of the signal and sensitive detection of target sequences. “

Thus for the reasons of record in view of the response to argument this rejection is maintained.

3. Claims 93 is rejected under 35 U.S.C. 103(a) as being unpatentable over Calzone et al (Methods in Enzymology (1987) volume 152, pages 611-632) and Clinical Micro Sensors, Inc (WO01/06016, Published January 25, 2001) as applied to claims 1-9, 11-18, 20-25, 43-45, 91-101, 109-116, 119-129 above, and further in view of Nikiforov et al (US Patent issued May 21, 1996).

The teachings of Calzone and CMS are set forth above in paragraph 2.

Calzone and CMS do not teach the use T7 exonuclease.

However, Nikiforov et al teaches the use of T7 exonuclease in primer extension assays to generate single stranded nucleic acids (see abstract). Nikiforov et al teaches that T7 exonuclease has the advantage over other nucleases that it has maximal activity in buffers suitable for DNA polymerase activity.

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to improve the Calzone and CMS electrochemical Invader based nucleic acid detection method by use of the T7 exonuclease taught by Nikiforov, because Nikiforov teaches T7 exonuclease can be used in the same buffer that amplification. The artisan would have a reasonable expectation of success as the artisan is substituting one known and characterized nuclease for another.

Response to Arguments

The response reiterates the arguments to the combination of Calzone and CMS and asserts the Nikiforov does not correct these deficiencies. These arguments have been thoroughly reviewed but are not considered persuasive as the arguments to Calzone and CMS have been addressed above and there are no specific arguments directed to the teachings of Nikiforov.

4. Claims 102-105 and 130-133 are rejected under 35 U.S.C. 103(a) as being unpatentable over Calzone et al (Methods in Enzymology (1987) volume 152, pages 611-632) and Clinical Micro Sensors, Inc (WO01/06016, Published January 25, 2001) as applied to claims 1-9, 11-18, 20-25, 43-45, 91-101, 109-116, 119-129 above, and further in view of Heller et al (US Patent 5,605,622, filed Issued February 25, 1997).

Clinical Micro Sensors is here after referred to as CMS.

The teachings of Calzone and CMS are set forth in paragraph 2 above.

Calzone and CMS do not teach the use of electrochemical technique utilizing selectively one or more electrodes functionally surrounded by permeable membrane that is permeable on the basis of charge, size, or hydrophobicity.

However, Heller et al teaches the use of permeation layers covering electrodes that allow solvent movement, while allowing exclusion based on size and charge (see column 11, lines 3-23; column 13, lines 30-55). Heller teaches the use of charge in the permeability layer he also inherently teaches the use of hydrophilic layers, as charge molecules are hydrophilic. Heller teaches the permeation layer functionally surrounding the electrode inhibits large proteins in the sample from binding the

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electrode, thus allowing the use of DC current (see column 11, lines 17-35). If the large proteins bound to the electrode, the large proteins would act as insulators, and cause a short circuit.

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve the method of Calzone and CMS to include the use of a electrode with a selectively permeable membrane (permeation layer) of Heller, because Heller teaches the permeation layer allows the use of direct current without the insulating effects of large proteins binding to the electrode. The use of Heller's permeation layer would thus result in more accurate and sensitive assays. The artisan would have a reasonable expectation of success as the artisan is replacing one electrode used for the detection of nucleic acid with another electrode that has also been used to detect nucleic acids.

Response to Arguments

The response reiterates the arguments to the combination of Calzone and CMS and asserts the Heller does not correct these deficiencies. These arguments have been thoroughly reviewed but are not considered persuasive as the arguments to Calzone and CMS have been addressed above and there are no specific arguments directed to the teachings of Heller.

5. Claim 19, 117, 118 are rejected under 35 U.S.C. 103(a) as being unpatentable over Calzone et al (Methods in Enzymology (1987) volume 152, pages 611-632) and Clinical Micro Sensors, Inc (WO01/06016, Published January 25, 2001) as applied to

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claims 1-9, 11-18, 20-25, 43-45, 91-101, 109-116, 119-129 above, and further in view of Hall et al (US Patent 5,994,069 issued November 30, 1999).

Clinical Micro Sensors is here after referred to as CMS.

The teachings of Calzone and CMS are set forth in paragraph 2 above.

Calzone and CMS do not teach the use of a second recognition cassette that is labeled for detection of the cleavage reaction of a first partially hybridized complex.

However, Hall et al teaches a method of signal amplification using an invader probe, an unlabeled 1st probe, and a labeled second probe (see figure 96, and column 71 lines 45-52). Hall teaches the invader probe binds a first target sequence, while the 1st probe partially hybridizes the target and the unhybridized 5' tail of the 1st probe is released. The 5' tail of the 1st probe than hybridize a second target sequence, causing the 5' tail of the 2nd labeled probe to only partially hybridize to the second target sequence. Hall teaches the labeled 5' end of the second sequence is thus cleaved, released, and detected. The 2nd labeled probe and 2nd target are a recognition cassette.

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improvement of the ETM invader method of Calzone and CMS by use of Halls 2nd labeled probe and target. The ordinary artisan would be motivated to improve CMS method by use of Hall's 2nd labeled probe and target, because Hall teaches it amplifies the signal, resulting in a more sensitive assay. The combined teachings of Calzone, CMS and Hall would result in a more sensitive electrochemical invader assay, than that taught by CMS. The artisan would have

reasonable expectation of success as the artisan is substituting one known method of nucleic acid detection for another.

Response to Arguments

The response reiterates the arguments to the combination of Calzone and CMS and asserts the Hall does not correct these deficiencies. These arguments have been thoroughly reviewed but are not considered persuasive as the arguments to Calzone and CMS have been addressed above and there are no specific arguments directed to the teachings of Hall.

Summary

No claims are allowed over prior art cited.

Conclusion

6. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to STEVEN C. POHNERT whose telephone number is (571)272-3803. The examiner can normally be reached on Monday-Friday 6:30-4:00, every second Friday off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, James (Doug) Schultz can be reached on 571-272-0763. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Steven Pohnert

/Juliet C Switzer/

Primary Examiner, Art Unit 1634